1932

Anatomical Study of Water Shoots and Ordinary Twigs

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ANATOMICAL STUDY OF WATER SHOOTS AND ORDINARY TWIGS

By

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A thesis submitted to the Committee on Advanced Degrees, South Dakota State College of Agriculture and Mechanic Arts, in partial fulfillment of the requirements for the degree of Master of Science.

July 1932

INTRODUCTION

Water shoots or long shoots are those which appear low on the trunks of trees, or about stumps of trees recently cut down. The ordinary twigs are those found at the ends of branches.

Interest in the comparative study of water shoots and ordinary shoots on trees was aroused by the very marked differences, even in a single year's growth, in length and diameter of the two types. The former is greater in diameter, much longer, and with more nodes, longer internodes and larger leaves than the twigs which grew ordinarily at the ends of branches.

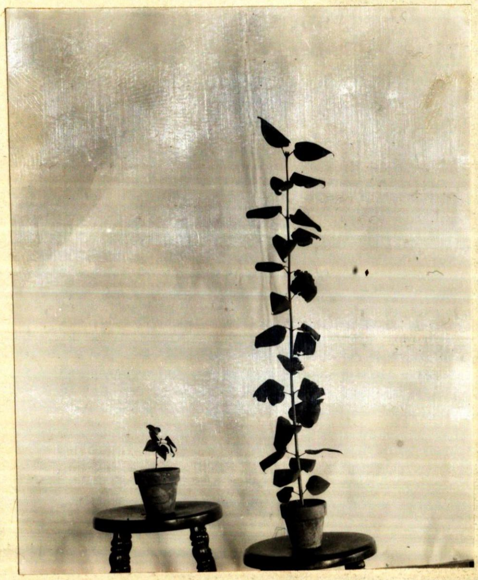

Fig. 1—Syringa vulgaris: Habit of water shoot (right) and ordinary shoot (left) X 1/10

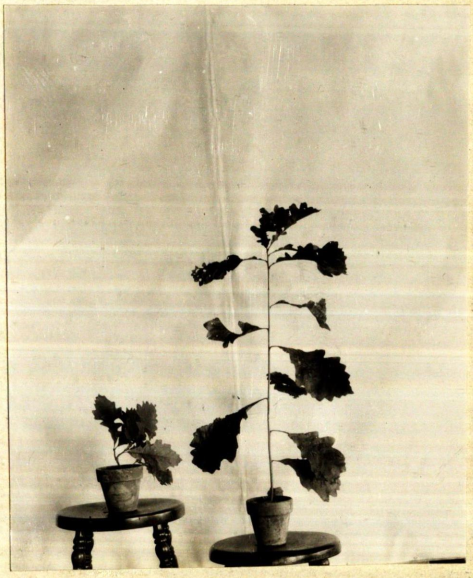

Fig. 2—Quercus sp.: Habit of water shoot (right) and ordinary shoot (left) X 1/10

Fig. 3--Elaeagnus angustifolia: Habit of water shoot (right) and ordinary shoot (left). X 1/8

The object of this investigation was to correlate gross differences with differences in microscopic anatomy and to see what developmental phenomena are responsible for the differences.

HISTORY

Bos (1) made a study of lateral twigs which grew on Picia excelsa and which had the same appearance as the main stem. The twig grew not from a dormant, but from an adventitious bud. He also described water shoots in Betula alba, Prunus cerasum, Robina psendococea, and Fagus sylvatica.

Rosenthaler (8) discussed water shoots with reference to Rupp's theory which concerns the ascent of sap in trees.

Johanson (5) also made a study of lateral shoots which appeared for several years on Platanus. They were found also to be produced from adventive buds.

Holmes (4) made a study of the anatomy of Hazel and Ash wood in reference to the conductivity of water. In selecting material he chose shoots developing from branches that had been pruned off the previous year. He found that the water shoots were much longer and thicker than the ordinary twigs, growing normally at the end of branches; that the leaves were much larger on the former and, even though vigorous vegetative branches developed, that no reproductive organs were formed. The leaves were found to be largest at the nodes in the lower middle of the water shoot. The internodes were longest in that region also. The water shoots had much larger piths than had ordinary shoots, the pith decreasing in diameter slightly toward the top and more rapidly toward the base. The amount of xylem was found to be greater toward the base with little change in the amounts of tissue outside the cambium. The wood cells near the tip were practically all capable of transmitting water but lower down the number of fibrous cells rapidly increased.

Reed (7), in a study of slow and rapid growths, dealt with terminal shoots on pruned and unpruned trees. In this investigation he found that those shoots developing on the pruned branches grew more rapidly than those on the unpruned branches. Reed concluded that the excessive growth of the former, however, was due to some catylitic process, which he does not explain.

Reed (6) also did some work with cognate shoots on lemon. Because of removal of the terminal bud, buds that otherwise would have remained dormant, were induced to grow. He found that those nearest the growing point began growth first and continued to develop more rapidly than those beginning later and farther removed from the top of the shoot. He explains the results of his observation by reason of there being a greater supply of appropriate food materials to the apical part of the shoot, or else a more rapid transformation of food materials, probably due to catylytic agents.

MATERIALS AND METHODS

Quericus sp, Elaeagnus angustifolia, and Syringia vulgaris were selected for comparison. They were near at hand and at the same time showed pronounced differences between the two kinds of shoots.

The first collection was made in the autumn of 1931 before the leaves had fallen. Both water shoots and short shoots, as taken from the same tree, were selected as near the average growth as it was possible to find.

The stems were first measured in length and diameter, the internodes counted and measurements made of the leaves.

In preparing the material for microscopic observation, short lengths were cut from the successive internodes and fixed in formalin acetic alcohol, treated with 15% hydrofluoric acid for three weeks then washed, dehydrated in alcohol, cleared in xylol and embedded in paraffin. Short shoots were sectioned transversally 10 micron with a rotary microtome, mounted and stained with safranin and light green. The material from the long shoots did not section well in paraffin and therefore was cut transversally by hand with a razor blade, dehydrated, stained as the short shoot sections and mounted.

This material was supplemented with a second collection of shoots of new growth late in the spring of 1932. Since there was less lignification at this time longitudinal sectioning was more satisfactory than it had been with older stems collected the previous year.

INVESTIGATION

To observe the extent of the region of growth in length, lilac stems of both long and ordinary growth were marked. Waterproof ink was used and marks were made 1 centimeter apart, from the growing point to the base of the shoot. After a period of three weeks the shoots were again measured.

The short shoot when first measured was 2 centimeters long and at the end of three weeks had grown 2 centimeters, doubling its length. The long shoot, which at first measured 23 centimeters, increased to 52 centimeters, or 2.28 times the first length.

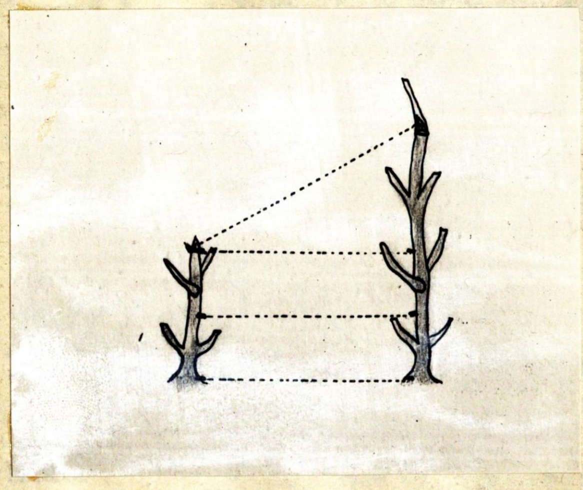

Fig. 4—Syringa vulgaris: Diagrams showing region of elongation in ordinary shoot. Actual size.

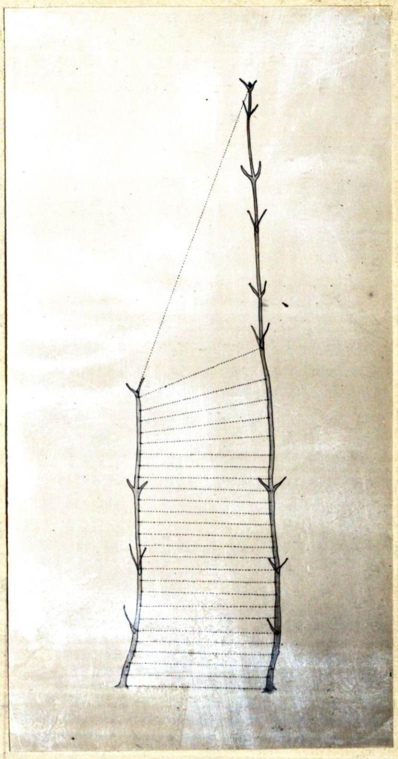

Fig. 5—Syringa vulgaris: Diagram showing region of elongation in the water shoot. X 1/5.

Figure 4 shows that elongation in the short shoot was confined to the apical centimeter and was practically all new growth; in the long shoot, however, (Figure 5) elongation occurred through the last 5 centimeters of the tip. These five centimeters were increased by 4.5 centimeters. The elongation of the half centimeter which extended beyond the last ink mark and which therefore included the growing point, increased in length 24.5 centimeters. The total elongation of the long shoot in three weeks time was 29 centimeters as compared with 2 centimeters increase in length of the short shoot in the same period of time.

The long Syringa shoot was found to be 10.35 times as long as the short one; 3.3 times as thick at the base, 2.3 times as thick at the tip with 13 more nodes; and with leaves that averaged 2.4 x 1.9 times as large as those on the short shoot. (Fig. 1)

The long oak shoot was found to be 20.8 times as long as the short one; only slightly wider in diameter at the base with 10 more nodes and with leaves 1.34 x 1.48 times as large as those of the short shoot. (Fig. 2)

The long shoot of Elaeagnus was 5.3 times as long as the short one; 4 times as wide at the base with 37 more nodes; and bearing leaves that were 2 times as wide but about the same length.

	Long Shoot				Short Shoot			
	Length	Diam.	No. of Nodes	Average Leaf Size	Length	Diam.	No. of Nodes	Average Leaf Size
Lilac	44	1/2	19	3 x 4½	4 1/4	1/8	6	1 1/4 x 2 1/4
Oak	27½	3/8	15	4¾ x 8½	1 3/8	2/8	5	3 1/2 x 5 3/4
Elaeagnus	45	1/2	62	2½ x 1¼	8 1/2	1/8	25	2 1/2 x 5/8

Table I—Gross dimensions in inches

Since there was such a variety of lengths in the long shoots produced on one tree, only one shoot as near the average as possible was selected and measurements made from it. The short shoots showed but little difference in length, so only those used for sectioning were measured for data in the above table. In all cases the long shoots were found to be much longer, with more numerous and longer internodes and bearing more and larger leaves than was true of the short shoots.

The tissue regions of the two kinds of shoots were measured in transverse sections and their measurements checked against longitudinal sections.

Rather than making sections of each internode, the lowest, highest, and medium internodes were selected in all cases.

All drawings, other than Figures 1 and 2 were made with the camera-lucida. The tissue regions were measured on the drawings and the results transposed from actual numbers to percentages of the radius. The results appear in Table II.

Long Shoots

Specimen	Inter.	% Cork	% Cort.	% Per.	% Ph.	% Xy.	% Pith
Lilac	Highest	5.28	15.49	3.52	3.52	31.69	40.49
	Middle	3.87	12.92	3.87	3.61	36.16	38.76
	Lowest	4.30	13.15	3.11	4.78	39.00	35.89
Oak	Highest	4.95	35.54	7.84	7.84	13.90	32.18
	Middle	5.34	26.33	4.93	16.46	13.16	34.15
	Lowest	7.18	24.55	5.68	13.00	30.07	23.75
Elaeagnus	Highest	3.16	12.00	4.43	6.42	20.00	54.43
	Middle	1.12	4.04	2.06	5.61	28.08	58.87
	Lowest	1.04	2.09	3.43	5.20	32.50	55.98

Short Shoots

Lilac	Highest	9.47	18.95	6.16	7.10	26.54	31.40
	Middle	8.15	13.11	4.19	6.18	28.27	39.31
	Lowest	7.35	12.34	5.02	5.02	20.73	53.51

Short Shoots (continued)

Specimen	Inter.	% Cork	% Cort.	% Per.	% Ph.	% Xy.	% Pith
Oak	Highest	6.36	31.86	6.36	9.55	17.19	50.95
	Middle	5.72	31.29	4.58	15.26	19.84	21.75
	Lowest	5.17	29.97	3.80	14.38	32.19	20.54
Elaeagnus	Highest	3.24	22.72	4.54	4.54	9.80	35.71
	Middle	2.61	9.77	5.34	8.07	30.00	44.30
	Lowest	4.00	13.75	5.71	6.85	37.15	33.71

Table II—Transverse dimensions of the tissue regions given in percentages of the radius.

In the long shoots there is a decrease in thickness of the cortex and pith regions from the tip to the base in all except Elaeagnus which shows pith percentages as nearly constant. Corresponding to the decrease in thickness of cortex and pith, there is an increase in the thickness of conducting tissues. The proportions of the various regions in the short shoot showed tendencies very similar to those in the long shoot except that in the long shoot the regions, especially conducting tissues, are noticeably wider, giving the long shoot its characteristic large diameter.

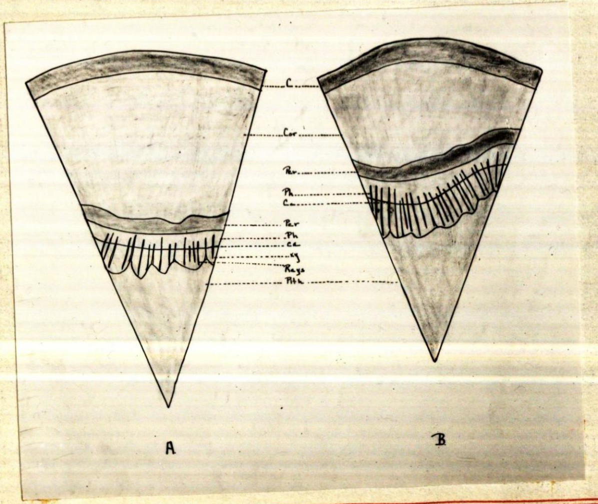

Fig. 6—Quercus sp: Diagramatic section of highest internode showing tissue regions; A, ordinary shoot; B, water shoot. X 30. C, cork; Cor., cortex; Per, pericycle; Ph, phloem; Ca, cambium; Xy, xylem; Rays; Pith.

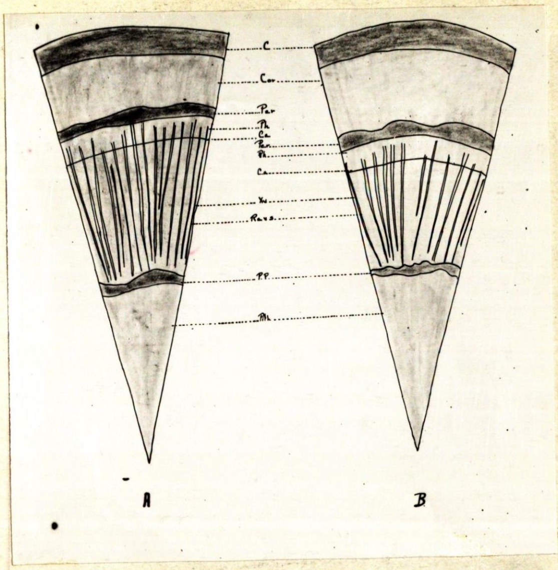

Fig. 7—Syringa vulgaris: Diagramatic section of highest internode showing tissue regions; A, ordinary shoot; B, water shoot. X 30
Legend same as for Fig. 6. PP, pitted pith cells.

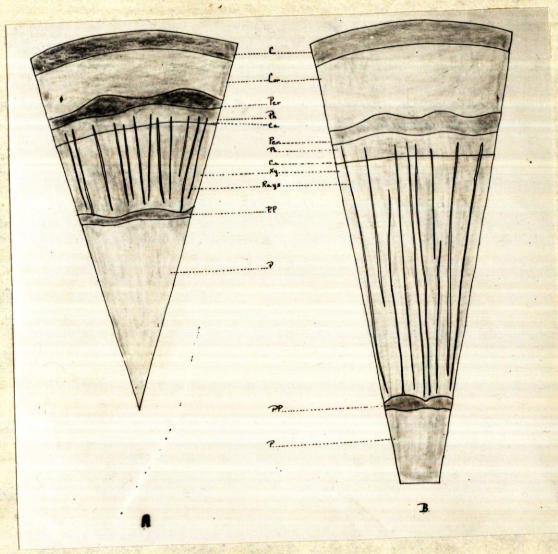

Fig. 8—Syringa Vulgaris: Diagramatic section of the lowest internode showing tissue regions; A, ordinary shoot; B, water shoot. X 30.
Legend as for Fig. 6.

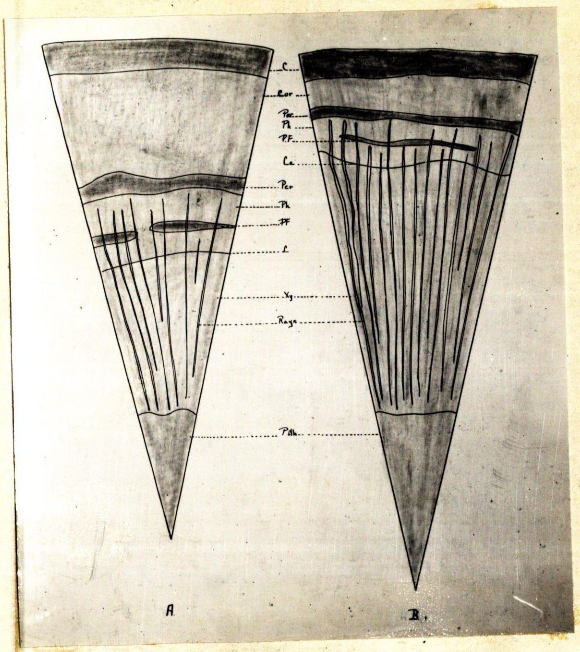

Fig. 9—Quercus sp: Diagramatic section of the lowest internode showing tissue regions; A, ordinary shoot; B. water short. X 23. Legend same as for Fig. 6.

Before studying the size of the cells in cross-sectioned material the microscope and camera-lucida were calibrated with a stage micrometer so that the magnifying power of various lense combinations was known.

Cells were picked at random, except that an effort was made to choose those of the size which appeared most frequently in order to get as near an average as possible. A number of cells of each tissue were drawn and an average taken of their dimensions. The results of measuring the three species, both long and short shoots, in all tissue regions have been collected in the following table. It is evident that the cork cells in all cases were larger in the short shoots. Two of the species, Quercus sp. and Elaeagnus angustifolia, showed smaller cells (with a few exceptions) in the long shoot than in the short shoot. Lilac, however, showed larger cells in the long than the short shoot with exception of the cork cells.

Long Shoots

	Inter.	Cork	Cortex	Peri.	Phloem	Xylem	Pith	P.ray	X. ray
Lilac	High	22 x 17	19 x 10	13 x 8	7 x 5	10 x 9	36 x 32	8 x 4	13 x 5
	Mid.	17 x 14	31 x 20	13 x 7	6 x 5	10 x 15	29 x 28	5 x 5	17 x 5
	Low.	23 x 14	19 x 12	13 x 7	7 x 8	12 x 12	37 x 36	7 x 5	15 x 7
Oak	High	13 x 9	17 x 12	5 x 5	8 x 4	5 x 4	12 x 10	11 x 6	7 x 5
	Mid.	8 x 4	9 x 7	5 x 5	4 x 3	5 x 4	9 x 8	7 x 3	7 x 3
	Low.	12 x 8	12 x 7	5 x 5	5 x 3	5 x 4	15 x 14	7 x 5	12 x 4
Elaeag.	High.	7 x 3	7 x 6	5 x 4	4 x 2	4 x 4	16 x 13	5 x 3	4 x 4
	Mid.	7 x 4	10 x 5	9 x 4	6 x 4	8 x 7	32 x 30	7 x 5	28 x 5
	Low.	6 x 3	11 x 5	8 x 5	6 x 4	9 x 7	17 x 7	7 x 5	30 x 4

Short Shoots

Lilac	High.	22 x 17	17 x 14	10 x 6	6 x 4	9 x 7	33 x 29	8 x 5	13 x 5
	Mid.	22 x 16	17 x 11	10 x 6	7 x 4	9 x 6	20 x 18	5 x 3	13 x 5
	Low	24 x 15	21 x 10	11 x 6	5 x 5	8 x 8	29 x 17	7 x 5	10 x 5
Oak	High.	15 x 9	14 x 9	9 x 6	9 x 7	5 x 5	28 x 15	16 x 8	8 x 7
	Mid.	13 x 12	12 x 7	4 x 4	8 x 5	19 x 17	16 x 16	9 x 5	8 x 4
	Low	17 x 8	14 x 9	7 x 6	10 x 4	10 x 8	18 x 17	8 x 5	9 x 8
Elaeag.	High.	5 x 4	8 x 7	7 x 6	4 x 4	5 x 4	17 x 14	4 x 4	5 x 3
	Mid.	9 x 5	13 x 8	10 x 5	6 x 4	9 x 9	24 x 20	8 x 5	15 x 5
	Low	15 x 4	22 x 8	10 x 7	9 x 5	7 x 7	23 x 20	9 x 7	13 x 5

Table III—Sizes of cells in long and short shoots in cross-section magnified 600 times and recorded in millimeters.

Similar examinations were made on the longitudinal sections and in these more variations were found, perhaps due to the difficulty of determining median sections of cells.

Measurements were made of the cortex, xylem, phloem, and pith cells. The main object in preparing these sections was to find whether the excessive length of the long shoots over the short ones was due to elongation of the cells or to a larger number of them. The results of this examination appear in the following table.

		Long Shoots				Short Shoots			
	Inter.	Cort.	Phloem	Xylem	Pith	Cort.	Phloem	Xylem	Pith
Lilac	High	14 x 15	35 x 5	36 x 6	20 x 13	34 x 15	38 x 6	51 x 9	21 x 11
	Mid.	20 x 14	58 x 5	76 x 7	17 x 7	24 x 13	81 x 7	75 x 9	27 x 25
	Low	22 x 13	82 x 8	78 x 11	27 x 22	19 x 17	70 x 6	80 x 8	27 x 17
Oak	High	17 x 10	54 x 6	67 x 8	16 x 7	22 x 14	50 x 9	59 x 9	16 x 12
	Low	31 x 17	74 x 8	63 x 7	20 x 16	21 x 15	77 x 8	99 x 9	16 x 15
Elaeag	High	15 x 9	30 x 5	69 x 6	17 x 15	12 x 10	37 x 7	77 x 9	16 x 13
	Mid.	37 x 17	44 x 8	96 x 8	27 x 25	40 x 20	46 x 10	61 x 12	56 x 32
	Low	66 x 17	78 x 9	96 x 9	56 x 35	45 x 28	59 x 7	87 x 7	38 x 27

Table IV—Sizes of cells in long section of long and short shoots magnified 600 times and recorded in millimeters.

From the table it is obvious that in the first internode in all cases the cells are larger in the short shoots, but beyond that the cells are larger in the long shoots. The greater difference is in the length of the cells.

Camera-lucida drawing of longitudinal sections of the highest internode of Oak (Fig. 10) and of Elaeagnus (Fig. 11) show the larger cells in the short shoot (A) as compared with the cells in the long shoot (B). A similar section of the lowest internode of lilac (Fig. 12) shows the typically larger cells in the long shoot (B) as compared with the smaller cells in the short shoot (A).

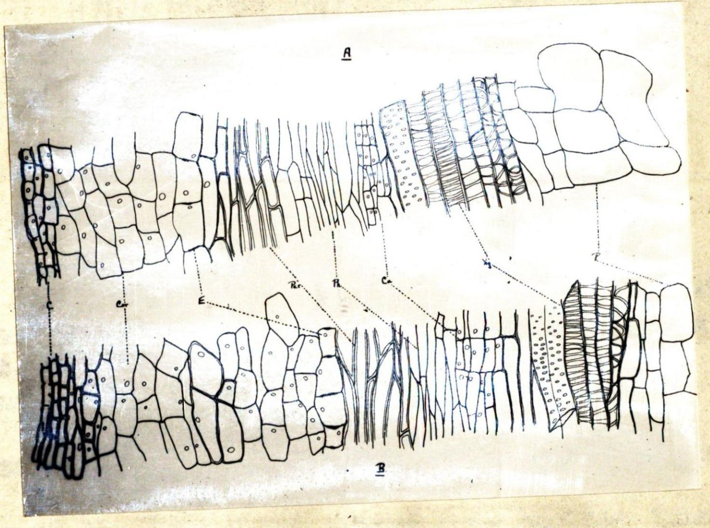

Fig. 10--Quercus sp: Drawing of highest internode in longitudinal section showing larger cells in A, ordinary shoot, than in B, the water shoot. X 100. C, cork; Cor, cortex; End, endodermis; Per, pericycle; Ph, phloem; Ca, cambium; Xy, xylem; P, pith.

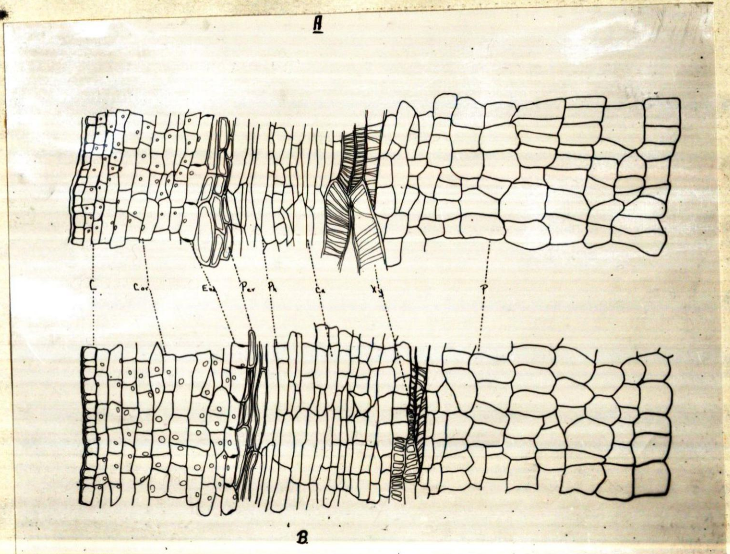

Fig. 11--Elaeagnus angustifolia: Drawing of highest internode in longitudinal section showing larger cells in the ordinary shoot A than the water shoot B. X 120. Legend same as for Fig. 10.

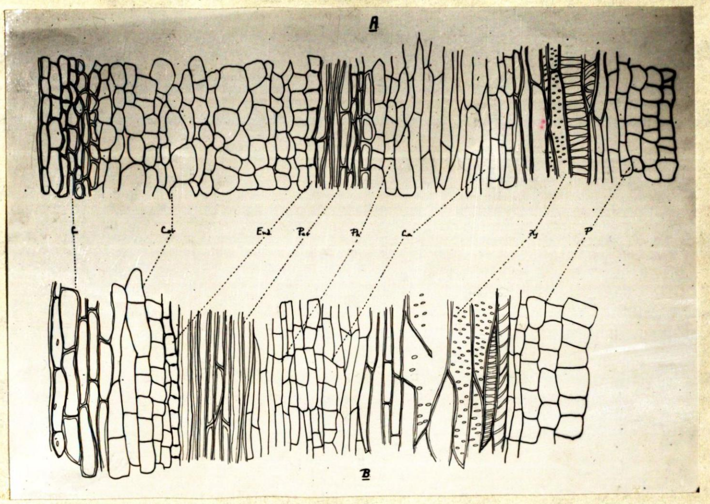

Fig. 12--Syringa vulgaris: Drawing of the lowest internode in longitudinal sections showing larger cells in the water shoot, B, than the short shoot, A. X 100. Legend same as for Fig. 10.

In examining the length of cortical, pith, and cork cells, it was found that those of the long shoot ranged from smaller than to 2.2 times (averaging 1.5 times) as long as those of the short shoot. Comparison of entire branch lengths revealed that in all three species the average length of long shoots was 15.5 times as great as the average length of the short shoots.

The lignification of cells in the various tissues seemed to be quite constant. There were a few variations but these were more pronounced between tip and base of the same shoot than in corresponding internodes of long and short shoots. The least differences were in the xylem and pericycle; they became more heavily lignified toward the base, especially in the long shoot.

The number of rays in secondary wood was also considered. A count of rays appearing in a specific arc revealed some variation. In lilac there were two times as many rays in the short shoot as in the long one. (Fig. 8)

This diagram shows the rays in both long and short shoots in the first internode. In Elaeagnus there were three-fourths as many in the short as long shoot. In Oak there were two-thirds as many in the long shoot as in the short one. (Fig. 9). The rays in the long shoot of Oak were found to be consistently one cell in tangential width as compared with those in the short shoot which were from one to three cells wide. The others showed one-celled rays with only a few appearances of two-celled rays in the lilac long shoot.

DISCUSSION

Observation of water shoots and ordinary shoots shows that the water shoots are much larger, have more internodes, bear larger leaves, and are wider in transverse section.

Upon microscopic examination of the tissue regions, it was found that in the long shoots there was a decrease in thickness of cortex and pith from apex to base, except for Elaeagnus, in which the pith remained fairly constant, and there was a corresponding increase in thickness of conductive tissue. The short shoots, with the excpetion of lilac, which increased its proportion of pith region, also showed an increase of conductive tissue from tip to base. These results check closely with the tissue regions as Holmes (4) described them in his work on Hazel and Ash wood.

An explanation of this difference was sought in microscopic examination of cell sizes. In all cases the cells in longitudinal sections were larger in the short shoot in the first internode. Below that they were larger in the long shoot, averaging 1.5 times as long as those in the short shoot. The long shoot in entirety averaged 15.5 times as long as the short shoots. But even though the cells were longer in the long shoot, their greater length does not alone explain the excessive length of the water shoots. The greater length of the water shoot is due not only to the larger size of cells but also to a greater number of them which implies increased activity on the part of the apical meristem. The formation of nodes is probably a factor controlled by hereditary influences and in a longer shoot there are necessarily more nodes and incidentally more leaves since leaves develop at the nodes. The size of the leaves is influenced by the same factor which influences its length. The greater length of the internodes is probably closely associated with the region of elongation. In the short shoot, the region of elongation was very short but in the long shoot the region of elongation was found to extend back five centimeters from the growing point and together with the larger number of cells is responsible for the greater length of the long shoot. In the long shoot, therefore, newly added cells continue to elongate for a longer time than they do in a short shoot.

Both meristematic activity and cell growth are dependent on water supply, and since the water shoot is in a more direct conduction line than the ordinary shoot, it is not surprising that the former exhibits more vegetative vigor. The wider diameter of the long shoot must also be due largely to the greater activity of a meristem, i. e., the cambium, since examination of cross-sectioned cells actually shows slightly larger cells in the short shoot, with one exception, (the lowest internode of the long lilac shoot) nor does the number of rays nor the tangential width of the rays give any explanation of the greater diameter, as the short shoot shows more rays than the long shoot. Although no explanation was found in this study for the development of the rays in this manner, there is a probability that the internodes were so short in the short shoots that some of the rays extended further than one internode and therefore overlapped one another. More would then appear in any given section of the short shoot.

The greater length and width of the long shoots then must be due to increased production of cells since cell sizes or ray size or number do not show sufficient evidence as sole influencing factors.

The excessive production of cells is probably due to some physiological reaction, which concerns the food and water supply, similar to the explanation which Reed (7) gave for the rapid growth of shoots developing near the tip of a pruned branch. Some reaction or catylitic agent is probably responsible for the conduction of food and water in larger amounts to regions of growth. The water shoot may be the result of growth of an adventive bud, as suggested by Johanson (5) which has been stimulated by injury to the stem or trunk and in some way interrupted the already organized semi-permeable tissue. Harvey (3) describes such an organization as being set up between two growing points as a result of the different electrical potentialities as compared with the other points on the stem or root. Placing the long shoot in a more direct connection with the water supply would make conditions more favorable for growth in the long shoot than in the short shoot. The short shoot is often far removed from the water supply, is exposed to more difficult conditions as more sunlight and wind, factors which enter into transpiration rates. The loss of water would cut down the amount utilized by the cells of the growing point and in this way so slow up their production.

The position of growth may also bear some relation to the lignification of the cells. It was found that there was little difference between wall thicknesses of cells of the corresponding internodes of long and short shoots except that lignification started closer to the tip of the short shoot, probably due to the slower rate of cell production than in the long shoot. The higher rate of transpiration in the short shoot may also be a factor. Toward the lowest internode of the long shoot more cells were found to be lignified than in the short shoot. Holmes also found this to be true, the lignification arising in response to greater stress toward the base of the shoot .

SUMMARY

In summing up the results of this investigation on comparing long and short shoots of oak, lilac and Elaeagnus it may be said that:

1. The long shoots were found to be longer, to have more and longer internodes, to bear larger leaves, and to be wider in diameter, than the short shoots. The differences in length of shoot, length and number of inter nodes are the result of increased activity of the apical meristem in the production of cells and the greater length of the region of elongation in the long shoot. The size in diameter is the result of greater activity on the part of the cambium in laying down secondary growth as the long shoot was also found to have a larger region of conducting tissue than the short shoot.

2. The region of elongation was found to extend over the last five centimeters in the long shoot as compared with the last half centimeter in the short shoot. The reason for this may be the rapid rate of cell formation in the long shoot and at the same time a greater supply of food and water at the growing point of the long shoot than in the short one.

3. Microscopic examinations showed in most cases a decrease in the pith and cortex regions and an increase in the proportion of water conducting tissue from tip to base. The short shoots showed a very similar development but to a less degree. The greater thickness of conductive tissue in the long shoot is due to an increased activity of the cambium.

4. Cell sizes were found to be larger in the short shoots in cross-section but longer in the long shoots beyond the highest internode. The difference in length at the first internode is probably due to a more rapid maturation of cells. The greater length of the cells in the long shoots and the narrower width may be due to the excessive supply of water. The factor of light as it is more often directly above the long shoots may be of some importance, the short shoots are more apt to be in a horizontal position and the light striking

them from the side.

5. The long shoot was found to have an increasing amount of lignification from tip to base, lignification starting closer to the tip in the short shoot, probably because of the short region of elongation than in the long yet not increasing as rapidly from tip to base. The increased formation of heavily lignified cells in the long shoot is probably due to a greater need of support in the long shoot than the short shoot.

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The writer wishes to take this opportunity to thank Dr. Ward L. Miller for suggesting this problem and for his interest and help in the work.

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